Alterations in Cardiovascular Responses of the Dog Following Rauwiloid®, An Alkaloidal Extract of *Rauwolfia serpentina.** (20919)

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Pharmacologic properties of hypotension, bradycardia and sedation, possessed by *Rauwolfia serpentina,* Benth. have aroused considerable clinical interest. Promising results have been reported from its use alone and in combination with other hypotensive agents in the management of hypertension (1-8). Rauwiloid, a mixture of alkaloids extracted from the root of *Rauwolfia serpentina,* has been demonstrated clinically and in the laboratory to possess the total activity of the root(2,6,9,10). At the recommended dosage, the effects of Rauwiloid are characterized by a slow onset of action, one to several weeks being required for them to be fully manifest (2,6). This latency is also observed in the laboratory animal wherein intravenous administration of doses even 50 times the clinical level produces a significant hypotension only after several hours. Since we were particularly interested in studying the effects of Rauwiloid at doses more approximate to the clinical level, it was not possible to perform such experiments by the usual method of observing the immediate effect vs. pre-medication control. In order, therefore, to emulate the therapeutic approach, the animals were medicated orally for several days.

The purpose of our experiments was to observe the effects of hypotensive doses of Rauwiloid on the cardiovascular responses of normotensive dogs. Such information might offer some insight into the mechanism of hypotension.

Method. Fifteen mongrel dogs were fed Rauwiloid orally for 5 days at a dose of 0.5 mg/kg/day.† This dose, although approximately 10 times the recommended clinical dosage, simply produced the picture of an increased clinical effect(9,10). On the sixth day the animals were anesthetized with pentobarbital sodium intravenously.‡ The trachea was intubated and 20 minutes allowed for stabilization of the anesthetic state. At this time, a femoral artery was cannulated for kymographic registration of blood pressure by a mercury manometer. The ipsilateral femoral vein was prepared for drug injection. After observations of blood pressure and heart rate had been made, epinephrine hydrochloride, acetylcholine chloride, histamine dihydrochloride and isopropylnorepinephrine hydrochloride (Isuprel) were injected intravenously at selected dosages.§ Each drug administration was flushed in with 1.0 ml normal saline solution. The animals were then subjected to a period of hypoxia, bilateral carotid occlusion, efferent and afferent vagal faradization. Observations were made on blood pressure during these procedures. The heart rate

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† A homogeneous mixture of Rauwiloid and U.S.P. Lactose at the ratio of 1:10 was prepared. The encapsulated dosage was administered to animals in meat once daily.

‡ In order to approximate an equal depth of anesthesia for each animal, the anesthetic, as a 6% solution in distilled water, was administered at the rate of 60 mg/minute until swallowing reflex was abolished.

§ Drugs, prepared as 0.001% solutions in physiologic saline administered to animals at following dose —μg/kg: Epinephrine hyrochloride—1 and 3, Acetylcholine hydrochloride—1, Histamine dihydrochloride —1, Isopropylnorepinephrine hydrochloride—.1, .5 and 1.0. Hypoxia induced by permitting animal to breathe 100% N_2 contained in rubber bag for 45 seconds. Respiratory mixture was delivered into endotracheal tube by means of a polarized flutter valve system. Then the carotid arteries were isolated and a 30 second carotid occlusion performed; then both vagi were severed and stimulated electrically using a Gorrell "Ene-Volt", the efferent end of the left vagus with 0.5 and 1 volt for 5 sec. and the afferent stump of the right vagus with 2 volts for 15 sec. An average of 15 minutes allowed to elapse between each of above procedures. Statistical significance was determined from the "t" value of the difference between the group means.

was also observed before and after each injection of Isuprel. A series of 15 dogs served as controls and were tested simultaneously and in the same manner as the medicated animals.

Results. The mean arterial blood pressure (MAP) and heart rate of the 15 anesthetized medicated dogs were 95 ± 12 (SD) mm Hg and 106 ± 24 beats/min. respectively. This compared with values of 128 ± 21 mm Hg and 146 ± 40 beats/min. for the control series. Eleven of the medicated animals appeared sedated.‖

The absolute blood pressure rises in response to epinephrine were greater ($p < 0.01$) in the treated animals. The peak pressure attained with the 1.0 μg dose was significantly higher ($p < 0.05$) in the medicated dogs; however, the difference of the peak pressures at the 3.0 μg level was barely non-significant, $0.06 > p > 0.05$ (Table I).

The absolute decrease in MAP and the nadirs of blood pressure produced by Isuprel were enhanced by Rauwiloid. The absolute heart rate increase was greater in the medicated group; however, the peak heart rates of the control and medicated series were not significantly different (Table I).

Fourteen of 15 medicated dogs showed a reversal of the usual pressor response to hypoxia, the mean decrease in MAP being 14 ± 17 mm Hg, whereas all control animals responded with a rise in MAP averaging 34 ± 23 mm Hg.

The blood pressure rise evoked by bilateral carotid artery occlusion was markedly depressed in the treated animals, the absolute rise in MAP being 9 ± 8 mm Hg as compared to 36 ± 23 mm Hg for the control dogs. This depression was greater ($p < 0.02$) than could be predicted on the basis of the lower pre-existing MAP of the medicated animals. By this formula,¶ the response of the medi-

TABLE I. Pressor Response to Epinephrine and Depressor and Cardio-accelerator Response to Isopropylnorepinephrine (Isuprel) in Dogs Anesthetized with Pentobarbital after 5-Day Rauwiloid or Placebo Medication—15 Animals in Each Series.

Drug	Series	Dose, μg/kg	Pre-existing MAP,* mm Hg	Max response pressures,* mm Hg	Diff.,* mm Hg	Pre-existing H.R.,* beats/min.	Peak H.R.,* beats/min.	Difference,* beats/min.
Epinephrine	Control	1.0	127 ± 20	159 ± 26	32 ± 11			
		3.0†	121 ± 15	190 ± 31	70 ± 21			
	Medicated	1.0	99 ± 13	177 ± 20	78 ± 18			
		3.0†	99 ± 9	214 ± 20	115 ± 15			
Isuprel	Control	.1	131 ± 18	119 ± 19	12 ± 7	155 ± 43	174 ± 43	19 ± 14
		.5	133 ± 18	95 ± 22	38 ± 12	156 ± 47	212 ± 42	56 ± 22
		1.0	133 ± 18	79 ± 18	54 ± 13	153 ± 48	232 ± 42	79 ± 32
	Medicated	.1	109 ± 18	87 ± 18	22 ± 12	110 ± 22	153 ± 32	43 ± 21
		.5	110 ± 19	52 ± 10	58 ± 14	112 ± 22	203 ± 42	91 ± 33
		1.0	110 ± 19	46 ± 7	64 ± 15	111 ± 20	218 ± 45	107 ± 34

* All figures with stand. dev.
† 10 animals tested at these doses.

‖ The dogs were lacking their usual spontaneous activity, but were aroused readily and were capable of locomotion.

¶ Magnitude of carotid sinus pressor reflex has been related to the pre-existing MAP by Prochnik *et al.* (16). The formula used was:

$$\frac{\text{Carotid Occlusion Response (mm Hg)} \times 100}{\text{Pre-existing MAP} - 60}$$

cated dogs was 21 ± 24% vs. 51 ± 38% for the control animals.

Faradization of the afferent stump of the right vagus nerve elicited a primary neurogenic blood pressure rise in all control animals averaging 48 ± 26 mm Hg. Only one of 15 medicated animals displayed this primary rise; the animals in this series responded with a mean decrease in MAP of 6 ± 6 mm Hg. The secondary humorally medicated rise(11) however, was essentially unaltered from the control.

The hypotensive responses to acetylcholine, histamine and efferent vagal faradization were unaffected by Rauwiloid.

Discussion. Augmentation of the pressor effect of epinephrine and of the depressor effect of Isuprel permits an interesting contrast. The first instance may be a function of the lower pre-existing MAP induced by Rauwiloid. However, despite this lower level of arterial blood pressure, the hypotensive effect of Isuprel was enhanced. Moe(12) has reported potentiation of the pressor response to epinephrine by tetraethylammonium (TEA). The observation was largely attributed to depression of vagal activity by TEA. Since our experiments have demonstrated the integrity of the efferent vagus, Rauwiloid may alter the activity of some other reflex pathway to augment the pressor response to epinephrine. Furthermore, the hypotensive response to Isuprel was enhanced in the face of unaltered cardio-accelerator activity. One explanation of this phenomenon is that Rauwiloid may affect the vasomotor center and/or its reflex pathways in such a manner as to prevent the usual response to acute blood pressure alterations.

Reversal of the blood pressure rise evoked by hypoxia has heretofore been accomplished in the dog by adrenergic blockade(13) or section of all 4 buffer nerves(14). The carotid sinus reflex may be depressed by similar means although such depression is also produced by ganglionic blocking agents(15) or a low pre-existing MAP(16). The augmentation of the response to epinephrine and the intact response to efferent vagal faradization eliminate adrenergic blockade and generalized ganglionic blockade respectively, as factors. Furthermore, the MAP of the medicated animals was not so depressed as to reduce the carotid artery reflex to the extent observed. On the other hand, blockade of the primary rise in blood pressure elicited by faradization of the afferent vagus indicates interference with sympathetic activity somewhere in the brain stem. That Rauwiloid may exert a specific depression of sympathetic ganglia likewise cannot be dismissed. Bein (17) has observed similar effects in cats anesthetized with dial-urethane, following intravenous administration of reserpine, a pure crystalline alkaloid from *Rauwolfia serpentina.*

That this autonomic blockade may be a progressive syndrome is indicated by experiments on 5 dogs which received a single dose of one mg/kg of Rauwiloid intravenously, resulting in hypotension when tested 24 hours later. Three of these animals displayed reversal of the pressor response to hypoxia and the primary rise to afferent vagal faradization was nearly eliminated. However, the carotid sinus reflex was not significantly altered. This latter observation is in agreement with clinical data citing maintenance of postural blood pressure reflexes following Rauwiloid (2,6-8). Such being the case, one could postulate depression of certain central autonomic activities (pressor responses of hypoxia and afferent vagal faradization) at low doses; at higher dosage, those central activities concerned with the carotid sinus reflex and/or the activity of the baroceptors may be depressed.

Summary. Rauwiloid, an alkaloidal extract representing the total activity of the root of *Rauwolfia serpentina,* Benth., was administered orally for 5 days to 15 normotensive dogs. The following observations (compared to 15 control animals) were obtained on the sixth day: 1. There was a significant decrease in MAP and heart rate. 2. Eleven of 15 animals were sedated. 3. The pressor response to epinephrine was augmented. 4. The hypotensive and cardio-accelerator effects of isopropylnorepinephrine (Isuprel) were enhanced. 5. The blood pressure rise generally evoked by hypoxia was reversed. 6. The carotid sinus pressor reflex

was diminished. 7. Blockade of the primary blood pressure rise elicited by faradization of the afferent vagus occurred; the secondary rise was essentially unaltered. 8. The hypotensive responses to acetylcholine, histamine and efferent vagal faradization were not altered.

1. Bhatia, B. B., *J. Indian Med. Assn.*, 1942, v11, 262.

2. Livesay, W. R., and Miller, S. I., *J. Pharmacol. Exp. Therap.*, 1954, v110, 33.

3. Kapur, R. D., *J. Indian Med. Assn.*, 1942, v11, 198.

4. Mazundar, D. C., and Mulchergi, K. H., *ibid*, 1950, v19, 362.

5. Vakil, R. J., *Brit. Heart J.*, 1949, v11, 350.

6. Wilkins, R. W., *Ann. Int. Med.*, 1952, v37, 1144.

7. ———, *Mississippi Doctor*, 1953, v30, 359.

8. Wilkins, R. W., and Judson, W. E., *New England J. Med.*, 1953, v24, 48.

9. Gourzis, J. T., and Bauer, R. O., unpublished observations.

10. Cronheim, G. E., Stipp, C. E., and Brown, W. L., *J. Pharmacol. Exp. Therap.*, 1954, v110, 13.

11. Taylor, R. D., Page, I. H., and Corcoran, A. C., *Arch. Int. Med.*, 1951, v88, 1.

12. Moe, G. K., *J.A.M.A.*, 1948, v137, 1115.

13. Dustin, E., Memorandum Report No. MCREXD 696-794 USAF: Materiel Command, Wright-Patterson Air Force Base (10 Feb. 1949).

14. Gellhorn, E., and Lambert, E. H., *Vasomotor System in Anoxia and Asphyxia*, Univ. of Illinois Press, 1939.

15. Acheson, G. H., and Moe, G. K., *J. Pharmacol. Exp. Therap.*, 1946, v87, 220.

16. Prochnik, G., Maison, G. L., and Stutzman, J. W., *Am. J. Physiol.*, 1950, v162, 553.

17. Bein, H. J., *Experientia*, 1953, v9, 107.

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Presence in Human Sera of Complement Fixing Antibodies to Virus of Sporadic Bovine Encephalomyelitis. (20920)

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The Department of Public Health, School of Veterinary Medicine, of the University of California routinely obtains blood samples from the personnel and students in the School. The sera are tested by complement fixation or agglutination for antibodies to various etiological agents of disease that are transmissible from animals to man. This program is expanded at times to encompass other agents that have not as yet been reported as causing infection in man. This paper is a preliminary report of the result of one such investigation involving the virus of sporadic bovine encephalomyelitis (SBE). The data reported here indicts SBE as yet another disease of animals whose causative agent may infect man. There has been little in the literature to indicate its role in animal disease until the past few years and no reports of infection in man. The disease in animals was first recognized in Iowa and reported by McNutt (1). SBE has been reported from only 11 states, but Menges, Harshfield, and Wenner (2) suggest that it is much more widespread.

Materials and methods. Seven sera from personnel in the School, were tested for complement-fixing (C-F) or agglutinating antibodies with 9 different antigens. In addition, a total of 481 human sera were tested for C-F antibodies to the SBE virus and to the Henzerling strain of *Coxiella burneti*. These same sera were tested by agglutination for antibodies to *Brucella*. The antigen of primary significance in this report, designated here as LMcN, was received from Dr. Sam C. Wong of Lederle Laboratories. It is an experimental, soluble, C-F antigen prepared from a 10% suspension of the infected yolk sac of embryonated eggs by ether extraction in the same manner as for the preparation of typhus vaccine. The organism used in preparing this antigen was isolated from the pleural exudate of a sick calf by Dr. S. H. McNutt. Subsequent to receiving this antigen from Dr.